DOCKET NO: PHRM0001-100/0008US **PATENT**
Serial No.: 09/750,373 **FILED: December 28, 2000**

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Please amend claims 1, 9, 16, and 29-31.

STATUS OF CLAIMS

Claim 1 **(currently amended)** An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence of ~~with at least 99% homology to~~ SEQ ID NO: 25.

Claims 2-6 **(canceled)**

Claim 7 **(original)** The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule is DNA.

Claim 8 **(original)** The isolated nucleic acid molecule of claim 1 wherein said nucleic acid molecule is RNA.

Claim 9 **(currently amended)** An expression vector comprising a the nucleic acid molecule of claim 1.

Claim 10 **(previously presented)** The expression vector of claim 9 wherein said nucleic acid molecule comprises SEQ ID NO:12.

Claim 11 **(canceled)**

Claim 12 **(original)** The expression vector of claim 9 wherein said vector is a plasmid.

Claim 13 **(original)** The expression vector of claim 9 wherein said vector is a viral particle.

Claim 14 **(original)** The expression vector of claim 13 wherein said vector is selected from the group consisting of adenoviruses, baculoviruses, parvoviruses, herpesviruses, poxviruses, adenoassociated viruses, Semlike Forest viruses, vaccinia viruses, and retroviruses.

Claim 15 **(original)** The expression vector of claim 9 wherein said nucleic acid molecule is operably connected to a promoter selected from the group consisting of simian virus 40, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney virus, cytomegalovirus immediate early promoter, Epstein Barr virus, rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine, and human metalothionein.

Claim 16 **(currently amended)** A host cell transformed with ~~an~~ the expression vector of claim 9.

Claim 17 **(original)** The transformed host cell of claim 16 wherein said cell is a bacterial cell.

Claim 18 **(original)** The transformed host cell of claim 17 wherein said bacterial cell is E. coli.

Claim 19 **(original)** The transformed host cell of claim 16 wherein said cell is yeast.

Claim 20 **(original)** The transformed host cell of claim 19 wherein said yeast is S. cerevisiae.

Claim 21 **(original)** The transformed host cell of claim 16 wherein said cell is an insect cell.

Claim 22 **(original)** The transformed host cell of claim 21 wherein said insect cell is S. frugiperda.

Claim 23 **(original)** The transformed host cell of claim 16 wherein said cell is a mammalian cell.

Claim 24 **(previously presented)** The transformed host cell of claim 23 wherein said mammalian cell is selected from the group consisting of chinese hamster ovary cells, HeLa cells, African green monkey kidney cells, human 293 cells, and murine 3T3 fibroblasts.

Claims 25 **(previously presented)** An isolated nucleic acid molecule comprising SEQ ID NO:12.

Claims 26-28 **(canceled)**

Claim 29 **(currently amended)** A composition comprising a nucleic acid molecule of ~~any one of claims~~ claim 1 or 25 and an acceptable carrier or diluent.

Claim 30 **(currently amended)** A composition comprising a the ~~recombinant~~ expression vector of claim 9 and an acceptable carrier or diluent.

Claim 31 **(currently amended)** A method of producing a polypeptide that comprises SEQ ID NO:25, said method comprising the steps of:

a) introducing ~~a recombinant~~ an expression vector of claim 10 into a compatible host cell;

b) growing said host cell under conditions for expression of said polypeptide; and

c) recovering said polypeptide.

Claim 32 **(original)** The method of claim 31 wherein said host cell is lysed and said polypeptide is recovered from the lysate of said host cell.

Claim 33 **(original)** The method of claim 31 wherein said polypeptide is recovered by purifying the culture medium without lysing said host cell.

Claim 34 **(withdrawn)** An isolated polypeptide encoded by a nucleic acid molecule of claim 1.

Claim 35 **(withdrawn)** The polypeptide of claim 34 wherein said polypeptide comprises a sequence selected from the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46.

Claim 36 **(withdrawn)** The polypeptide of claim 34 wherein said polypeptide comprises an amino acid sequence homologous to a sequence selected from the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46.

Claim 37 **(withdrawn)** The polypeptide of claim 34 wherein said sequence homologous to a sequence selected from the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46 comprises at least one conservative amino acid substitution compared to the sequences in the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46.

Claim 38 **(withdrawn)** The polypeptide of claim 34 wherein said polypeptide comprises a fragment of a polypeptide with a sequence selected from the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46.

Claim 39 **(withdrawn)** The polypeptide of claim 34 wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:25 and SEQ ID NO:46.

Claim 40 **(withdrawn)** A composition comprising a polypeptide of claim 34 and an acceptable carrier or diluent.

Claim 41 **(withdrawn)** An isolated antibody which binds to an epitope on a polypeptide of claim 34.

Claim 42 **(withdrawn)** The antibody of claim 41 wherein said antibody is a monoclonal antibody.

Claim 43 **(withdrawn)** A composition comprising an antibody of claim 41 and an acceptable carrier or diluent.

Claim 44 **(withdrawn)** A method of inducing an immune response in a mammal against a polypeptide of claim 34 comprising administering to said mammal an amount of said polypeptide sufficient to induce said immune response.

Claim 45 **(withdrawn)** A method for identifying a compound which binds nGPCR-x comprising the steps of:

a) contacting nGPCR-x with a compound; and

b) determining whether said compound binds nGPCR-x.

Claim 46 **(withdrawn)** The method of claim 45 wherein the nGPCR-x comprises an amino acid sequence selected from the group consisting of SEQ ID NO:25 and SEQ ID NO:46.

Claim 47 **(withdrawn)** The method of claim 45 wherein binding of said compound to nGPCR-x is determined by a protein binding assay.

Claim 48 **(withdrawn)** The method of claim 45 wherein said protein binding assay is selected from the group consisting of a gel-shift assay, Western blot, radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis, and ELISA.

Claim 49 **(withdrawn)** A compound identified by the method of claim 45.

Claim 50 **(withdrawn)** A method for identifying a compound which binds a nucleic acid molecule encoding nGPCR-x comprising the steps of:

a) contacting said nucleic acid molecule encoding nGPCR-x with a compound; and

b) determining whether said compound binds said nucleic acid molecule.

Claim 51 **(withdrawn)** The method of claim 50 wherein binding is determined by a gel-shift assay.

Claim 52 **(withdrawn)** A compound identified by the method of claim 50.

Claim 53 **(withdrawn)** A method for identifying a compound which modulates the activity of nGPCR-x comprising the steps of:

a) contacting nGPCR-x with a compound; and

b) determining whether nGPCR-x activity has been modulated.

Claim 54 **(withdrawn)** The method of claim 53 wherein the nGPCR-x comprises an amino acid sequence selected from the group consisting of SEQ ID NO:25 and SEQ ID NO:46.

Claim 55 **(withdrawn)** The method of claim 53 wherein said activity is neuropeptide binding.

Claim 56 **(withdrawn)** The method of claim 53 wherein said activity is neuropeptide signaling.

Claim 57 **(withdrawn)** A compound identified by the method of claim 53.

Claim 58 **(withdrawn)** A method of identifying an animal homolog of nGPCR-x comprising the steps:

a) comparing the nucleic acid sequences of the animal with a sequence selected from the group of sequence consisting of SEQ ID NO:11 to SEQ ID NO:13, and SEQ ID NO:45, and portions thereof, said portions being at least 10 nucleotides; and

b) identifying nucleic acid sequences of the animal that are homologous to said sequence selected from the group sequence consisting of SEQ ID NO:11 to SEQ ID NO:13, and SEQ ID NO:45, and portions thereof.

Claim 59 **(withdrawn)** The method of claim 58 wherein comparing the nucleic acid sequences of the animal with a sequence selected from the group of sequences consisting of SEQ ID NO:11 to SEQ ID NO:13, and SEQ ID NO:45, and portions thereof, said portions being at least 10 nucleotides, is performed by DNA hybridization.

Claim 60 **(withdrawn)** The method of claim 58 wherein comparing the nucleic acid sequences of the animal with a sequence selected from the group of sequences consisting of SEQ ID NO:11 to SEQ ID NO:13, and SEQ ID NO:45, and portions thereof, said portions being at least 10 nucleotides, is performed by computer homology search.

Claim 61 **(withdrawn)** A method of screening a human subject to diagnose a disorder affecting the brain or genetic predisposition therefor, comprising the steps of:

(a) assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression, or biological activity of at least one nGPCR that is expressed in the brain, wherein the nGPCR comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:12, and SEQ ID NO:45, and allelic variants thereof, and wherein the nucleic acid corresponds to a gene encoding the nGPCR; and

(b) diagnosing the disorder or predisposition from the presence or absence of said mutation, wherein the presence of a mutation altering the amino acid sequence, expression, or biological activity of the nGPCR in the nucleic acid correlates with an increased risk of developing the disorder.

Claim 62 **(withdrawn)** A method according to claim 61, wherein the nGPCR is nGPCR-1002 comprising an amino acid sequence set forth in SEQ ID NO:46 or an allelic variant thereof.

Claim 63 **(withdrawn)** A method according to claim 61, wherein the nGPCR is nGPCR-1007 comprising an amino acid sequence set forth in SEQ ID NO:25 or an allelic variant thereof.

Claim 64 **(withdrawn)** A method according to claim 61, wherein the disease is a mental disorder.

Claim 65 **(withdrawn)** A method according to claim 61, wherein the assaying step comprises at least one procedure selected from the group consisting of:

a) comparing nucleotide sequences from the human subject and reference sequences and determining a difference of either

at least a nucleotide of at least one codon between the nucleotide sequences from the human subject that encodes an nGPCR-1002 allele and an nGPCR-1002 reference sequence, or

at least a nucleotide of at least one codon between the nucleotide sequences from the human subject that encodes an nGPCR-1007 allele and an nGPCR-1007 reference sequence;

(b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences;

(c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and

(d) performing a restriction endonuclease digestion to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences.

Claim 66 **(withdrawn)** A method according to claim 65 wherein the assaying step comprises: performing a polymerase chain reaction assay to amplify nucleic acid comprising nGPCR-1002 or nGPCR-1007 coding sequence, and determining nucleotide sequence of the amplified nucleic acid.

Claim 67 **(withdrawn)** A method of screening for an nGPCR-1002 or nGPCR-1007 hereditary mental disorder genotype in a human patient, comprising the steps of:

(a) providing a biological sample comprising nucleic acid from said patient, said nucleic acid including sequences corresponding to alleles of nGPCR-1002 or nGPCR-1007; and

(b) detecting the presence of one or more mutations in the nGPCR-1002 allele or the nGPCR-1007 allele;

wherein the presence of a mutation in a nGPCR-1002 allele or nGPCR-1007 allele is indicative of a hereditary mental disorder genotype.

Claim 68 **(withdrawn)** The method according to claim 67 wherein said biological sample is a cell sample.

Claim 69 **(withdrawn)** The method according to claim 67 wherein said detecting the presence of a mutation comprises sequencing at least a portion of said nucleic acid, said portion comprising at least one codon of said nGPCR-1002 or nGPCR-1007 alleles.

Claim 70 **(withdrawn)** The method according to claim 67 wherein said nucleic acid is DNA.

Claim 71 **(withdrawn)** The method according to claim 67 wherein said nucleic acid is RNA.

Claim 72 **(withdrawn)** A kit for screening a human subject to diagnose a mental disorder or a genetic predisposition therefor, comprising, in association:

(a) an oligonucleotide useful as a probe for identifying polymorphisms in a human nGPCR-1002 gene or a human nGPCR-1007 gene, the oligonucleotide comprising 6-50 nucleotides in a sequence that is identical or complementary to a sequence of a wild type human nGPCR-1002 or nGPCR-1007 gene sequence or nGPCR-

1002 or nGPCR-1007 coding sequence, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution; and

(b) a media packaged with the oligonucleotide, said media containing information for identifying polymorphisms that correlate with mental disorder or a genetic predisposition therefor, the polymophisms being identifiable using the oligonucleotide as a probe.

Claim 73 **(withdrawn)** A method of identifying a nGPCR allelic variant that correlates with a mental disorder, comprising the steps of:

(a) providing a biological sample comprising nucleic acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny;

(b) detecting in the nucleic acid the presence of one or more mutations in an nGPCR that is expressed in the brain, wherein the nGPCR comprises an amino acid sequence selected from the group consisting of SEQ ID NO:25, and SEQ ID NO:46, and allelic variants thereof, and wherein the nucleic acid includes sequence corresponding to the gene or genes encoding nGPCR;

wherein the one or more mutations detected indicates an allelic variant that correlates with a mental disorder.

Claim 74 **(withdrawn)** A method according to claim 73, wherein the disorder is a mental disorder, and wherein the at least one nGPCR is nGPCR-1002, nGPCR-1007, or an allelic variant thereof.

Claim 75 **(withdrawn)** A purified and isolated polynucleotide comprising a nucleotide sequence encoding a nGPCR-1002 or nGPCR-1007 allelic variant identified according to claim 74.

Claim 76 **(withdrawn)** A host cell transformed or transfected with a polynucleotide according to claim 75 or with a vector comprising the polynucleotide.

Claim 77 **(withdrawn)** A purified polynucleotide comprising a nucleotide sequence encoding nGPCR-1002 or nGPCR-1007 of a human with a mental disorder;

wherein said polynucleotide hybridizes to the complement of SEQ ID NO:12 or of SEQ ID NO:45 under the following hybridization conditions:

(a) hybridization for 16 hours at 42EC in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate and

(b) washing 2 times for 30 minutes at 60EC in a wash solution comprising 0.1x SSC and 1% SDS; and

wherein the polynucleotide that encodes nGPCR-1002 or nGPCR-1007 amino acid sequence of the human differs from SEQ ID NO:25 or SEQ ID NO:46 by at least one residue.

Claim 78 **(withdrawn)** A vector comprising a polynucleotide according to claim 77.

Claim 79 **(withdrawn)** A host cell that has been transformed or transfected with a polynucleotide according to claim 77 and that expresses the nGPCR-1002 or nGPCR-1007 protein encoded by the polynucleotide.

Claim 80 **(withdrawn)** A host cell according to claim 79 that has been co-transfected with a polynucleotide encoding the nGPCR-1002 or nGPCR-1007 amino acid sequence set forth in SEQ ID NO:25 or SEQ ID NO:46 and that expresses the nGPCR-1002 or nGPCR-1007 protein having the amino acid sequence set forth in SEQ ID NO:25 or SEQ ID NO:46.

Claim 81 **(withdrawn)** A method for identifying a modulator of biological activity of nGPCR-1002 or nGPCR-1007 comprising the steps of:

a) contacting a cell according to claim 79 in the presence and in the absence of a putative modulator compound;

b) measuring nGPCR-1002 or nGPCR-1007 biological activity in the cell;

wherein decreased or increased nGPCR-1002 or nGPCR-1007 biological activity in the presence versus absence of the putative modulator is indicative of a modulator of biological activity.

Claim 82 **(withdrawn)** A method to identify compounds useful for the treatment of a mental disorder, said method comprising the steps of:

(a) contacting a composition comprising nGPCR-1002 with a compound suspected of binding nGPCR-1002 or contacting a composition comprising nGPCR-1007 with a compound suspected of binding nGPCR-1007;

(b) detecting binding between nGPCR-1002 and the compound suspected of binding nGPCR-1002 or between nGPCR-1007 and the compound suspected of binding nGPCR-1007;

wherein compounds identified as binding nGPCR-1002 or nGPCR-1007 are candidate compounds useful for the treatment of a mental disorder.

Claim 83 **(withdrawn)** A method for identifying a compound useful as a modulator of binding between nGPCR-1002 and a binding partner of nGPCR-1002 or between nGPCR-1007 and a binding partner of nGPCR-1007 comprising the steps of:

(a) contacting the binding partner and a composition comprising nGPCR-1002 or nGPCR-1007 in the presence and in the absence of a putative modulator compound;

(b) detecting binding between the binding partner and nGPCR-1002 or nGPCR-1007;

wherein decreased or increased binding between the binding partner and nGPCR-1002 or nGPCR-1007 in the presence of the putative modulator, as compared to binding in the absence of the putative modulator is indicative a modulator compound useful for the treatment of a mental disorder.

Claim 84 **(withdrawn)** A method according to claim 82 or 83 wherein the composition comprises a cell expressing nGPCR-1002 or nGPCR-1007 on its surface.

Claim 85 **(withdrawn)** A method according to claim 84 wherein the composition comprises a cell transformed or transfected with a polynucleotide that encodes nGPCR-1002 or nGPCR-1007.

Claim 86 **(withdrawn)** A method of purifying a G protein from a sample containing said G protein comprising the steps of:

a) contacting said sample with a polypeptide of claim 1 for a time sufficient to allow said G protein to form a complex with said polypeptide;

b) isolating said complex from remaining components of said sample;

c) maintaining said complex under conditions which result in dissociation of said G protein from said polypeptide; and

d) isolating said G protein from said polypeptide.

Claim 87 **(withdrawn)** The method of claim 86 wherein said sample comprises an amino acid sequence selected from the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46.

Claim 88 **(withdrawn)** The method of claim 86 wherein said polypeptide comprises an amino acid sequence homologous to a sequence selected from the group of sequences consisting of SEQ ID NO:24 to SEQ ID NO:27, and SEQ ID NO:46.

Claim 89 **(withdrawn)** The method of claim 86 wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:25 and SEQ ID NO:46.